

Thermo Scientific NK1:NFATc1 Redistribution[®] Assay

The Redistribution technology monitors the cellular translocation of GFP-tagged proteins in response to drug compounds or other stimuli and allows easy acquisition of multiple readouts from the same cell in a single assay run. In addition to the primary readout, high content assays provide supplementary information about cell morphology, compound fluorescence, and cellular toxicity.

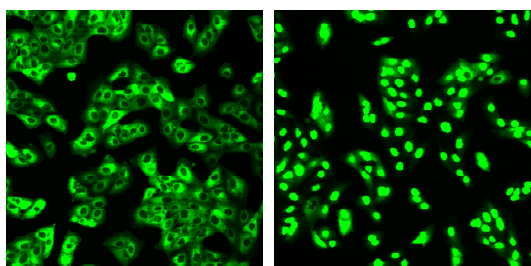


Figure 1. Nuclear translocation of EGFP-NFATc1. Cells expressing the NK1 receptor were treated with 10 nM substance P for 30 min (right panel) or untreated (DMSO control, left panel). Activation of the receptor leads to nuclear translocation of EGFP-NFATc1, which can be detected by the image analysis algorithm.

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Neurokinin-1 (NK1) receptor belongs to the group of tachykinin receptors and is stimulated by substance P. NK1 receptor activation has been associated with a number of neurological diseases such as depression. In this assay, the NK1 receptor has been transfected into the GPCR Reporter Assay for Gq-coupled Receptors, where receptor activation leads to release of cytoplasmic Ca^{2+} , which in turn induces NFATc1 (nuclear factor of activated T-cells) translocation. Binding of an agonist to the extracellular parts of NK1 receptor causes a conformational change in the receptor. This leads to conformational changes in heterotrimeric G proteins at the intracellular face of the receptor, exchange of GDP for GTP on the alpha subunit ($G\alpha_q$) and subsequent release of $G\alpha_q$ from the β - γ subunit. GTP-bound $G\alpha_q$ then activates phospholipase C, which catalyzes the formation of DAG and IP3 from PIP2. Free IP3 diffuses into the cytoplasm, and activates IP3 receptors on the endoplasmic reticulum (ER) resulting in Ca^{2+} release

from the ER into the cytoplasm. NFATc1 is a transcription factor involved in T-cell signaling and tissue development, and its activity is controlled by the Ca^{2+} /Calmodulin-dependent phosphatase, calcineurin. Inactive NFATc1 resides in the cytosol, but in response to elevated calcium levels, NFATc1 is dephosphorylated by calcineurin, followed by rapid translocation to the nucleus [1,2].

Features

- Designed to assay compounds for their ability to activate the NK1 receptor
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

Highlights:

- **Biologically relevant data**
Compounds tested in a cellular environment
- **Validated**
Functionally tested cells provided with an optimized assay protocol
- **Easy to use**
Just plate cells, add compounds, and image

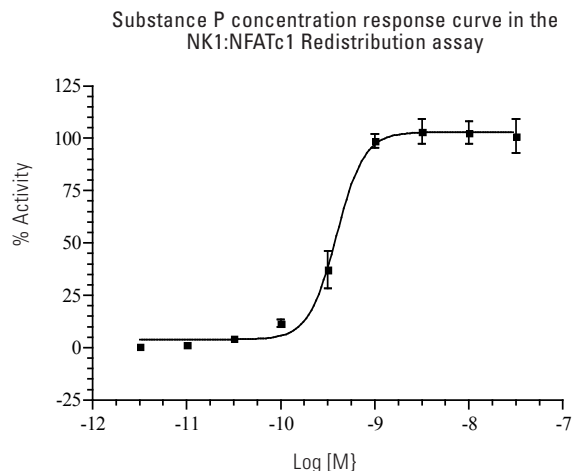


Figure 2. Substance P concentration response curve in the NK1:NFATc1 assay. The EC_{50} of substance P is ~400 pM. Concentration response was measured in 9 point half log dilution series ($n = 8$). Cells were treated with substance P for 30 min. Cells were then fixed and nuclear translocation was measured using the Cellomics ArrayScan V^{TI} Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (10 nM substance P) and negative control (0.25% DMSO).

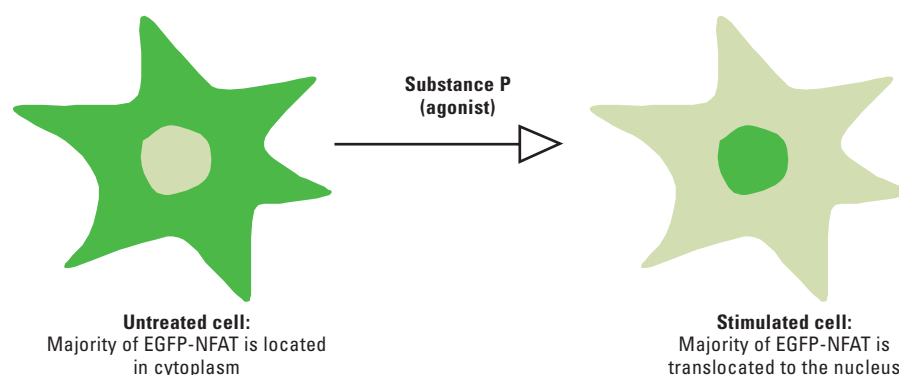


Figure 3. Illustration of the NFAT translocation in the NK1:NFATc1 assay.

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Assay Details

Recombinant U2OS cells stably expressing human neurokinin-1 (NK1) receptor and human NFATc1 fused to the C-terminus of enhanced green fluorescent protein (EGFP). The NK1:NFATc1 assay is designed to screen for agonists causing translocation of EGFP-NFATc1 to the nucleus. Test compounds having activity in the assay are considered to be agonists for NK1 receptor activation. Substance P is used as reference compound in the assay. The NK1:NFATc1 assay is validated with an average $Z' = 0.78 \pm 0.06$, suitable for both screening and profiling applications.

Imaging

The translocation of EGFP-NFATc1 can be imaged on most HCS platforms and fluorescence microscopes. The filters should be set for Hoechst (350/461 nm) and GFP/FITC (488/509 nm) (wavelength for excitation and emission maxima). Consult the instrument manual for the correct filter settings. The translocation can typically be analyzed

on images taken with a 10x objective or higher magnification.

The primary output in the NK1:NFATc1 Redistribution assay is the translocation of EGFP-NFATc1 from the cytoplasm to the nucleus. The data analysis should therefore report an output relating to the GFP fluorescence intensities in the nucleus and the cytoplasm.

Imaging on Thermo Scientific Cellomics ArrayScan V^{TI}

This assay has been validated on the Cellomics Arrayscan V^{TI} using a 10x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the Redistribution V3 BioApplication. The output used was MEAN_CircRingAvgIntenRatioLog (Log of the ratio of average fluorescence intensities of nucleus and cytoplasm (well average)). The minimally acceptable number of cells used for image analysis in each well was set to 200 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.

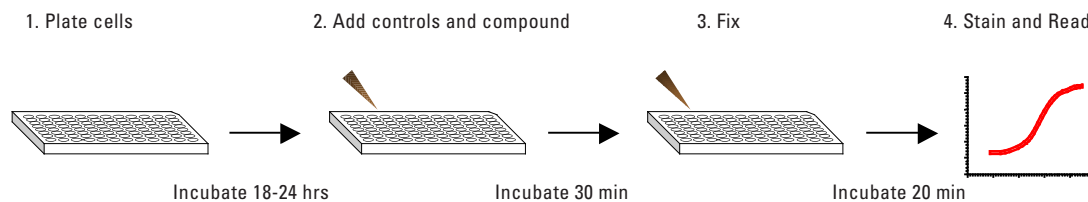


Figure 4. The NK1:NFATc1 Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
048_01	NK1:NFATc1 Redistribution Assay	U2OS	•	•	

The Redistribution Assays are available in 3 product formats, Profiling, Screening and CryoRedi, for different volume and level of convenience needs. The Redistribution Assays can also be accessed through the Thermo Scientific Managed Services.

Related Thermo Scientific Products

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO-K1	•	•	
K0100111	Cellomics NFAT-1 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100041	Cellomics p38 MAPK Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404601	Cellomics Cell Cycle I HCS Reagent Kit	Antibody- and dye-based reagent kit			
CX03004-INS	Cellomics ONE BioApplication Suite	High content data acquisition and analysis software			
CX03102A/B	Cellomics ArrayScan V ^{TI}	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

References

1. Rao, A. et al. *Annu. Rev. Immunol.*; 15, 707-747, 1997.
2. Masuda, E.S. et al. *Cell Signal.*; 10, 599-611, 1998

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